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COMPARATIVE INITIATION/PROMOTION SKIN PAINT STUDIES OF B6C3F$_1$ MICE, SWISS (CD-1®) MICE, AND SENCAR MICE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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ABSTRACT

In 1983, an *ad hoc* panel on chemical carcinogenesis testing and evaluation recommended additional methods that should be used by the National Toxicology Program (NTP) for the detection and evaluation of chemical carcinogens. One recommendation was that there should be an increased emphasis on short-term tests to detect agents that do not exert genetic effects such as some promoting agents.

Initiation/promotion models have been used routinely to identify chemicals with promoting potential and to study tumorigenesis. In one model, a topical subcarcinogenic dose of a chemical is first applied to the back of the skin (initiation) followed by repeated topical applications of one or more chemicals (promotion) and the skin is monitored for tumor development. Mouse skin has been shown to be more responsive (i.e., develops tumors using this protocol) than other commonly used laboratory rodent models. However, not all mouse strains are equally sensitive.

The skin tumor response of the $B6C3F_1$ mouse using the initiation/promotion protocol was not known. Since the $B6C3F_1$ mouse is commonly used in NTP carcinogenesis studies and much is known of its biology and response to chemical carcinogens, known initiators and promoters were used to compare the tumor response sensitivity of $B6C3F_1$ mouse skin to that of two often-used responsive strains, Swiss (CD-1®) and SENCAR mice. The combination of 7,12-dimethylbenz(a)anthracene (DMBA) initiation and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion was selected because this pair is routinely used to study tumorigenesis. However, DMBA requires metabolic activation to achieve initiation and it was possible that the $B6C3F_1$ mouse metabolism might not make this conversion (DiGiovanni and Juchau, 1980). Therefore, a second study was conducted using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), a direct acting carcinogen, as the initiator. MNNG is not used as frequently for mouse skin studies as is DMBA. In addition to the promoter TPA, benzoyl peroxide (BPO), a non-phorbol ester and known promoter after DMBA initiation, was also used (Slaga *et al.*, 1981). Each initiating chemical was used in combination with each promoting chemical as described on the following page.

Additional groups of male and female mice of each strain were treated with repeated applications of acetone (vehicle control), repeated applications of promoter (TPA or BPO) without prior initiation treatment (promoter reference controls), or a single application of the initiator (DMBA or MNNG) followed by repeated applications of acetone (initiator controls)

All three strains of mice demonstrated sensitivity by developing skin tumors after topical application of the chemicals under study (DMBA, MNNG, TPA, and BPO). The most sensitive of the three strains appeared to be SENCAR mice, in the sense that lower doses of the test chemical were generally required to produce effects equivalent to those in the other two strains. Skin tumors also tended to develop earlier and with greater multiplicity in SENCAR mice than in the other two strains. By these criteria, the overall sensitivity of Swiss (CD-1®) mice was intermediate, and $B6C3F_1$ mice showed the least overall sensitivity to dermal carcinogenicity.

In response to recommendations regarding specific short-term tests and also on the skin tumor response sensitivity of various initiators and promoters, SENCAR mice would be the most acceptable strain to use for such studies. Though the $B6C3F_1$ mice were less responsive in the skin initiation/promotion protocol, promotion data from this strain may, at times, be of more use in explaining mechanisms of tumor development (e.g. when there is a strain-specific response observed in 2-year carcinogenicity studies or effects on melanocytes are suspected).

Study Design for the 1-Year Comparative Initiation/Promotion Skin Paint Studies

Initiation/Promotion[a]	Mouse Strain		
	$B6C3F_1$	Swiss (CD-1®)	SENCAR
Design A			
0.25 μg DMBA/TPA[b]		X	X
2.5 μg DMBA/TPA	X	X	X
25 μg DMBA/TPA	X	X	X
50 μg DMBA/TPA	X		
2.5 μg DMBA/BPO[c]	X	X	X
25 μg DMBA/BPO	X	X	X
Design B			
100 μg MNNG/TPA	X	X	X
1,000 μg MNNG/TPA	X	X	X
100 μg MNNG/BPO	X	X	X
500 μg MNNG/BPO	X	X	X
1,000 μg MNNG/BPO	X	X	X
Complete Carcinogen[d]			
2.5 μg DMBA/2.5 μg DMBA	X	X	X
100 μg MNNG/100 μg MNNG	X	X	X

[a] Mice received a single initiating application followed by repeated promotion applications for up to 52 weeks.
[b] $B6C3F_1$ and Swiss (CD-1®) mice received 5 μg TPA; SENCAR mice received 1 μg TPA.
[c] BPO applications contained 20 mg BPO.
[d] Mice received repeated applications of DMBA and MNNG.

The 1-year complete carcinogen studies used repeated applications of low concentrations of the carcinogens DMBA and MNNG. The skin tumor response in all three strains under these conditions was more similar than in the initiation and promotion studies. There was a high incidence of skin tumors in all three strains with both carcinogens. More $B6C3F_1$ and SENCAR mice developed skin tumors and averaged more tumors per mouse than did Swiss (CD-1®) mice. Skin tumors developed earlier in SENCAR mice than in $B6C3F_1$ and Swiss (CD-1®) mice. Although $B6C3F_1$ mice exhibited the lowest overall sensitivity to the initiation/promotion protocol when compared to Swiss (CD-1®) and SENCAR mice, the response of $B6C3F_1$ mice was similar to Swiss (CD-1®) and SENCAR mice for complete carcinogen studies.

A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 8.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on the comparative initiation/promotion skin paint studies on June 21, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have four major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, and
- to judge the significance of the experimental results by scientific criteria.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 21, 1994, the draft Technical Report on these comparative initiation/promotion skin paint studies of $B6C3F_1$ mice, Swiss (CD-1®) mice, and SENCAR mice received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. W.C. Eastin, NIEHS, introduced the report by relating that in 1983, an *ad hoc* panel on chemical carcinogenesis testing and evaluation commissioned by the NTP had recommended that the NTP increase emphasis on short-term tests to detect agents that do not exert genetic effects such as some promoting agents. For the $B6C3F_1$ mouse, the strain commonly used for NTP carcinogenesis studies and for which a large database exists, the skin tumor response using the initiation protocol was not known. Therefore, the objectives of this research project were to compare the tumor response sensitivity of $B6C3F_1$ mouse skin to that of two often-used responsive strains, Swiss (CD-1®) and SENCAR mice, using known chemical initiators and promoters, as well as complete carcinogens.

Dr. Eastin described the study design and techniques used for in-life data collection for these 1-year studies and provided a detailed report of the study results. For the initiation/promotion studies, all three strains of mice demonstrated sensitivity by developing skin tumors after topical applications of the chemicals under study (DMBA, MNNG, TPA, and BPO). At the concentrations tested, the most sensitive of the three strains appeared to be SENCAR mice, in the sense that lower doses of test chemical were generally required to produce effects equivalent to the other two strains. Skin tumors also tended to develop earlier and to exhibit increased multiplicity in SENCAR mice relative to the other two strains. By these criteria, the overall sensitivity of Swiss (CD-1®) mice was intermediate, and $B6C3F_1$ mice showed the least overall sensitivity to dermal carcinogenicity. In the complete carcinogen studies, the skin tumor response in all three strains was more similar than in the initiation/promotion studies. There was a high incidence of skin tumors in all three strains with both carcinogens. More $B6C3F_1$ and SENCAR mice developed skin tumors and averaged more tumors per mouse than did Swiss (CD-1®) mice. Skin tumors developed earlier in SENCAR mice than in $B6C3F_1$ and Swiss (CD-1®) mice.

Dr. Ryan, a principal reviewer, suggested that there should be some discussion regarding the increased sensitivity of the SENCAR strain in terms of survival in the TPA/TPA promoter reference group and whether this complicated the statistical analyses. Dr. Eastin explained that many of these animals were not really dying but were being removed from the study after lesions had developed. Dr. Ryan also questioned the implication of tumors appearing in the groups receiving TPA without DMBA or MNNG initiation. Dr. Eastin said there should have been tumors only in groups receiving initiation with promotion or those receiving repeated application of carcinogens (DMBA or MNNG). Dr. Ryan also asked why a standard survival analysis on time to tumor was not done. Dr. J.K. Haseman, NIEHS, responded that the analysis was based on the time of appearance of the first tumor, an in-life observation.

Dr. Bailey, the second principal reviewer, noted that, as stated in the report, these studies were designed to provide mechanistic tumorigenesis data and to determine if this model would be a useful adjunct to the NTP toxicity/carcinogenesis studies. Dr. Eastin responded that the NTP wanted the Subcommittee's advice on whether this model was a useful adjunct. Dr. Bailey said there should be a statement in the front of the report that the most sensitive strains of mice to tumor promotion were also those that were significantly more sensitive to the irritant effects of the chemicals as evidenced by a marked inflammatory reaction.

Dr. Miller, the third principal reviewer, said that there was a need for explanation of the possible effects of dose errors in the DMBA/TPA promoter reference group upon the study results. Dr. Eastin noted that the correct dose was given for 50 of the 52 weeks, so he doubted that the error would have affected the outcomes. Dr. Miller thought that the effect on the findings of the much lower dose of TPA promoter in SENCAR mice as compared with the other two strains should be discussed. Dr. Miller asked for a clearer explanation of why this study was

conducted and what conclusions can be drawn about performing such studies in $B6C3F_1$ mice.

Dr. Ryan moved that the Technical Report on comparative initiation/promotion skin paint studies of $B6C3F_1$ mice, Swiss (CD-1®) mice, and SENCAR mice be accepted. Dr. Bailey seconded the motion, which was accepted unanimously with eleven votes.

Regarding the usefulness of the initiation/promotion model for providing mechanistic data as an adjunct to the NTP toxicology and carcinogenesis studies, Dr. Reddy said that with limited resources, the NTP should not be doing initiation/promotion studies on most test chemicals. Dr. Bailey thought that there had been a forum or review of this subject several years ago by the Environmental Protection Agency. Dr. R. Griesemer, NIEHS, said that this was correct and there was also a review by the International Agency for Research on Cancer that dealt with initiation/promotion in all organs where data existed, not just skin. The newer approaches to understanding cell cycle stage specificity might diminish priority for standard initiation/promotion studies. Dr. Klaassen said use of this protocol would need to be more selective and based on some scientific rationale, for example, as with chemicals associated with thyroid tumors that act through a promotional mechanism. Dr. Klaassen cautioned that a major goal of toxicology was not to find the most sensitive test or species but rather the species or test most predictive for humans. Dr. Reddy commented that most promoters were organ specific. Dr. G. Lucier, NIEHS, stated that the NTP would like to be able to select from a variety of possibilities, including initiation/promotion, transgenic mice, mechanistic studies of chemical interactions with receptors or target genes, or alternative methods, with the ultimate goal being to develop information that will be more predictive of what might happen in humans.